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Gamma-Radiation Induced Endoreplication in Exposed CHO Cell Line

Parinaz Mehnati

Department of Medical Physics, School of Medicine and Drug Applied Research Center,
Tabriz University of Medical Sciences, Tabriz, Iran

Abstracts: Several studies have highlighted the possibility of radiation induced chromosomal aberrations. The present study explains the susceptibility of numerical and structural chromosomal alterations, specifically endoreplication in irradiated Chinese Hamster Ovary (CHO) cell line. A total of 426 metaphase spreads prepared from CHO cells, exposed to 2 or 6 Gy of ^{60}Co γ-rays were analyzed. The frequency of dicentric chromosomes was 48 %, endoreplications 12.6 % and acenteric fragments 12.7 %. The manifestation of endoreplication after exposure to ionizing radiation in comparison with control cells was significant. Also, dicentric chromosomes were appeared to be created in a dose-dependent manner and observed more frequently than acentric fragments. However, the dose-dependent effect of irradiation was not seen in the case of endoreplication. This study suggests the possible hazardous effect of radiation on spindle function, microtubule motor or structure, resulting in endoreplication.

Key words: Gamma-Radiation · Endoreplication · CHO Cell · Chromosomal Aberrations

INTRODUCTION

Radiation biology has been challenged with the genetic effects of radiation exposure in recent years. These effects are attributed to the immediate, direct and targeted action of radiation on DNA. In the initiation stage of ionizing, radiation causes molecular damage by modification of the primary structure of DNA due to the passage of the particle through the cell. The initial radiation damage to the DNA structure could lead to specific biological lesion, such as chromosomal aberration, genetic instability and mutation in a specific somatic cell target [1]. Molecular damage is the result of deposition of the energy and production of ionized and excited state of the molecule's physical events, radical species and other molecular products in the environment of chemical events of the DNA. Goss and Harris [2] were described that ionizing radiation breaks chromosomes into smaller fragments with sizes that are proportional to the radiation dose. They explained that the closer two genes are together more likely to segregate within a segment of DNA following treatment with ionizing radiation. Studies on animal cells have frequently shown that exposure of cells to ionizing radiation causes dose-dependent increase in the incidence of numerical [3, 4] and structural chromosome anomalies [5, 6]. A variety of cell types has been used to study the radiation-induced chromosomal damage and a cell dependent pattern of aberrations has been detected. However studies on potential induced numerical and structural chromosome anomalies in transmission to the next generations are appear to be inadequate [7].

Duplication is a chromosomal rearrangement that results in an increase in the copy number of a particulate chromosomal region, but endoreplication or endoreduplication is the replication of DNA during S phase of the cell cycle without subsequent completion of mitosis or cytokinesis. It is one of the numerical chromosomal abnormalities and referred to duplicated number of all chromosomes including in respected cell.

A chromosomal base study on cultured human lymphocytes showed that a single dose of radiation causes the eventual endoreplication of many lymphocytes even from any stage of interphase [8]. Although many of

Corresponding Author: Parinaz Mehnati, Department of Medical Physics,
School of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran.
Tel/Fax: +98-411-3364660.

mammalian malignant cells also replicate the chromosomes without mitosis, the function seems different from endoreplication. The project on the function of endoreplication-dependent genes and nuclear proteins in plants identify genes and proteins specifically accumulated in endoreplication nuclei using genome-wide screening methods [9]. You might imagine that if gene copy number is important in the duplicated region, this duplication can cause phenotypic consequences. Also, Weber and Hoegerman [8] showed colcemid or radiation could induce endoreduplication in BUdR-labeled human lymphocytes. We examined control and exposed cells with optimized colchicine concentration and treatment time for determining its suspicious effects to cause endoreduplication. A phenomenon was observed that frequency of aberration induced radiation in exposed cells to 2 Gy dose of radiation because it is routine dose for cancer treatment and 6 Gy for investigation of increasing dose effects. The objective of this investigation is to evaluate the endoreduplicated chromosomal aberration exposed to γ-rays.

MATERIALS AND METHODS

Chinese hamster ovary cell line (CHO-K1) provided by National Cell Bank of Iran (NCBI), Pasteur Institute of Iran. The cells were grown in monolayer in Ham's F12 medium (Sigma) supplemented with 10% fetal calf serum. Asynchronous cells, were equally divided in 12 culture flasks (25 cm^2) including: 4 cultures were treated with 2 Gy of ^{60}Co γ-rays (Theratronics 1000), 4 cultures were exposed to 6 Gy and the remaining 4 cultures were left without treatment (negative control) at the concentration of 10^3 cells/ml for each flask. The experiments were repeated 3 times using CHO cells irradiated at 2 or 6 Gy. Cultures were maintained in incubator at 37°C in a humidified atmosphere of 5% CO_2 until the mitotic cells were harvested after 72 hours. We experimented control and exposed cells with changing in concentration of colchicines within the range between 0.01 and 1µg/ml during one to three hours of treatment to establish dubious effects of colchicines to cause endoreduplication. The cultured cells were subjected to hypotonic treatment with 75 mM of KCL for 20 min and then fixed in a 1:3 mixture of methanol-acetic acid for 10 min.

Then, one drop of suspension cells placed on the glass microscope slides from around 30 cm to spread which have been in -20°C before. Cells stained with Giemsa 1 % solved in buffer and they were analyzed under a light microscope for detecting chromosomal aberration specifically endoreplication per individual CHO cells [10]. Mitogene was not used in this assay as the cells have the potential of normal division. We used Mann-Whitney U, Chi square test for statistical analysis.

RESULTS

The examples of metaphase spread chromosome aberrations in exposed CHO cells to γ-radiation are shown by Figure 1. They were observed with light microscopy and by photographs. The frequency of numerical and structural chromosome anomalies presented in diverse aberration cell. This photo showing an increase of chromosomes number from 19 for control cells to 36 is an avouchment example of endoreplication in irradiated cells.

The 72 hours post radiation treatment cultures indicated that the frequency of dicentric chromosomes after exposure to 2 Gy or 6 Gy was 17.4% or 30.6% (Table 1) and for endoreplication it was 7% or 5.6% (Table 2) respectively in contrast to control cells. One hundred metaphase of control cells were observed for comparison. Endoreduplication was not observed in the control cells but one dicentric and one fragment were detected. However, in comparison with control cells, the manifestation of endoreduplication frequency in exposed cells to 2 Gy or 6 Gy was significant ($P = 0.006$, $P = 0.01$) respectively.

Other types of aberrations such as acentric fragment, ring, three centeric and diverse were shown from table 3 to table 5. Percentage of acentric fragment in exposed cells to 2 or 6 Gy was 5.6% or 7.1% respectively (Table 3). The proportion of acentric fragment was not equal to dicentric.

The frequency of ring and centeric aberrations was adequate and explained with table 4. About 10.8 % of 426 recorded cells were included multiple aberrations which named divers aberration (Table 5). In these cells between two and five aberrations observed per cell chromosomal analysis.

Also, the correlation between radiation dose and the type of chromosome aberrations for dicenteric, acentric fragment and diverse was demonstrated in Table (6). Only dicentric chromosomes showed significant dose-dependency. However, the dose-dependency was not found in the case of endoreplication.

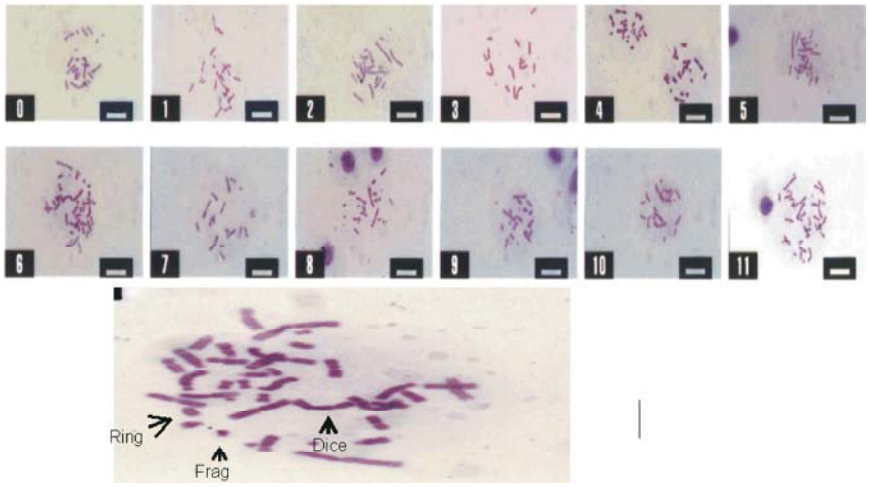

Fig. 1: The photos from 0 to 11 are metaphase spread chromosomes of CHO cells exposed to γ-radiation. An example of diverse Aberration cells. Including Dice (dicenteric), Frag (acentric fragment), Ring and Endoreplication (number of chromosomes is 38 counted per cell nuclei).

Table 1: Frequency of dicenteric aberrations per CHO cells

Dose	Aber/cell*	Frequency	Percent
	0	190	82.6
2Gy	1	40	17.4
	0	136	69.4
6Gy	1	56	28.6
	2	4	2

*: It means number of aberrations per each cell.

0: no aberration observed, 1: one aberration observed, 2: two aberration observed

Table 2: Frequency of endoreplication aberrations per CHO cells

Dose	Aber/cell*	Frequency	Percent
2Gy	0	214	93
	1	16	7
6Gy	0	185	94.5
	1	11	5.6

*: It means number of aberration per each cell. 0: no aberration observed, 1: one aberration observed

Table 3: Frequency of acentric fragment aberrations per CHO cells

Dose	Aber/cell*	Frequency	Percent
2Gy	0	217	94.3
	1	10	4.3
	2	3	1.3
6Gy	0	182	92.9
	1	11	5.6
	2	2	1
	3	1	0.5

*: It means number of aberrations per each cell

0: no aberration observed, 1: one aberration observed, 2: two aberration observed and 3: three aberration observed

Table 4: Frequency of centeric and ring aberrations per CHO cells

Dose	Aber/cell*	Frequency	Percent
2Gy	0	228	99.1
	1	2	0.9
6Gy	0	195	99.5
	1	1	0.5

*: It means number of aberration per each cell. 0: no aberration observed, 1: one aberration observed

Table 5: Frequency of diverse aberrations per CHO cells

Dose	Aber/cell*	Frequency	Percent
2Gy	0	218	94.8
	1	9	3.9
	1.5	1	0.4
	2	2	0.9
6Gy	0	185	94.4
	1	8	4.1
	1.5	1	0.5
	2	1	0.5
	3	1	0.5

*: It means number of aberration per each cell.

0: no aberration observed, 1: two aberration observed, 1.5: three aberration observed

2: four aberration observed, 3: five aberration observed

Table 6: Mean (SD) of number of aberration separated by type and dose

	Dose	
Type of Aberration	2 Gy	6Gy
Dicentric	0.17 (0.37)	0.32 (0.51)
Acenteric fragment	0.06 (0.30)	0.91 (0.36)
Diverse	0.06 (0.28)	0.07 (0.33)

Optimal concentration of colchicines was 0.1 µg/ml during three hours which confirmed by negative control cells without endoreplication. This value was added to each flask prior to harvest.

DISCUSSION

Endoreplication is a phenomenon in which cells of higher eukaryotes replicate the chromosomes without intervening mitosis. The cells, as the result of endoreplication, contain several sets of chromosomes in the single nuclei. Radiation induced carcinogenesis and mutagenesis are stochastic effects. One of the important signs of radiation carcinogenesis on the cell is chromosomal aberration involving numerical and structural changes [8]. Also, Effects of gamma radiation on cell cycle and division especially mitotic index have been observed [9].

The most prevalent abnormality after exposure referred by other researcher is dicenteric but in the present study, we demonstrate that endoreplication is notable as a post radiation chromosomal aberration in comparison with control cells (Table 2). This data presented colchicine treatment in different concentrations and time for taking optimal range without endoreplication in negative control cells. It cleared contravention effects of radiation with colcemid induce endoreplication referred before by Weber and Hoegerman [10].

The frequency of dicentric chromosomes in CHO cells induced ionizing radiation found to agree level with the corresponding value of other researcher results 72 hours post radiation treatment [11]. The chromosome analysis indicates that most of dicentrics produced consists of those without fragments, similar observations have also reported [12- 14]. In the yeast cells exposed to X-ray spindle pole bodies were duplicated as well forming the complete 1-μm spindle like as control yeast cells but nuclei in giant cells have lost the elongation ability and remain in a normal G_2-phase, thus preventing nuclear as well as cellular division [15]. Also, Agrawal *et al*. [16] found that, the cytogenetic effects of deltamethrin on rat bone marrow reduplication of chromosomes is most demotic abnormality. We used simple and carefully Giemsa staining assay for detection of some numerical and structural chromosomal alteration. The comparison of some chromosomal aberration frequency with Giemsa or FISH painting method does not give a statistical difference using irradiated lymphocytes [17], it is predominant in our present interested number of chromosomes data about endoreplication too.

Ionizing radiation induces a variety of DNA lesions such as base changes, strand breaks and DNA-protein cross-links [18]. These lesions have been thought to cause the important biological effects, including chromosome aberrations, cell death, gene mutation and malignant transformation. The model is based on the assumption that aberrations arise from clustered DNA lesions. The lesions are distributed in the cell nucleus according to the radiation track structure [19]. The data suggest percentage of reduplicated chromosome could mean duplicate gene may perform ancestrally conserved, redundant functions in cells. Because there is genetic and cytogenesis evidence indicates that some mutations map to reduplicated chromosomal regions [20]. There is evidence that X -ray-induced delayed cell death, delayed giant cell formation and delayed chromosome aberrations in normal human embryo cells. Also, in the our previous study, we observed individual CHO cells pre and post exposure to ionizing radiation for 5 or 7 generations (high and low LET). We found detail information about division delay, reproductive and interphase death, giant cell and lethal sectoring ratios in exposed cells [21, 22]. This study suggests cytogenetic effect of radiation especially endomitotic endoreduplication of chromosomes may be within distortion on the microtubular/mitotic spindle constitution. For more explaining of chromosome aberration results the cell cycle variation in exposed CHO cells to γ-radiation should be studied in the future.

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